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Relationship Between ApoE Gene Polymorphism and Cerebrovascular Disease in Qinghai Tibetan Population

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Abstract: To investigate the correlation between ApoE gene polymorphism and cerebral infarction (CI) and cerebral hemorrhage (ICH) in Tibetan patients with cerebrovascular disease, and the distribution of ApoE genotype in Tibetan nationality. We collected 94 patients as the experimental group, which hospitalized in Qinghai Provincial People's Hospital, Guoluozhou People's Hospital and Qinghai University Affiliated Hospital, including 48 cases of cerebral infarction (mean age 61.39 ± 10.48 years); 46 cases of cerebral hemorrhage (mean age 63.17 ± 10.92 years), and 96 healthy Tibetan residents from the physical examination center of Qinghai Provincial People's Hospital as control group. The results showed that in In the Tibetan population, the CI group was the most common in the ε3 alleles, with 48.0%, followed by ε2 (37.5%) alleles, the rarest of which was ε4 (14.6%). The most common one in ICH group were ε2 (43.5%), ε3 (45.7%) alleles, and the rarest one was ε4 (10.9%). ε3 was the most common allele in patients with Tibetan cerebrovascular disease. In the normal control group, ε2 (49.0%) was the most common alleles, followed by ε4 (33.3%), and ε3 (17.7%). ε3 allele may be a predisposing factor for cerebrovascular disease in Tibetan population. In Tibetan population, the majority alleles of ApoE were heterozygous E2/E3 and E2/E4, suggesting that hypoxia environment may be beneficial. The TG values in Tibetan populations varied among different alleles, suggesting that different alleles may influence lipid metabolism.

Keywords: Apolipoprotein E, Polymorphism, Cerebrovascular Disease

1. Introduction

The ApoE gene is one of the most important genetic factors for blood cholesterol levels. 14% to 16% of cholesterol variation is due to ApoE gene polymorphism. In the central nervous system, ApoE is involved in neuronal lipid metabolism, calcium transport, and signal transduction and so on [1]. The survey study found that ApoE has obvious racial differences. In China, the correlation has also been investigated between Naxi, Yi, Bai in Yunnan Province and Xinjiang Uygur [2-4]. The results showed that there were some differences among the ethnic groups in China. In addition, the study also found that there was closely related between ApoE gene polymorphisms and cerebrovascular disease. For example, ApoE gene polymorphism and stroke showed that ε3 was a common disease-causing allele (83% in patients group, 88% in control group), followed by the ε4 alleles (11% and 6.8% respectively) and the 2 alleles (6% and 5.2% respectively) in the Greek [5]. Qinghai Province is located in the Qinghai Tibet Plateau in western of China, and Tibetan residents have long lived there, cerebrovascular disease had been brought a heavy burden to Tibetan families. The purpose of this study is to clarify the correlation between Tibetan cerebrovascular disease and ApoE gene polymorphism and the distribution of ApoE genotype in Tibetan nationality.

2. Materials and Methods

Inclusion criteria: All included cases were from Department of Neurology in Qinghai Provincial People's Hospital, Affiliated Hospital of Qinghai University, Guoluo People's Hospital between February to December 2016. All the patients enrolled in the experimental group met the diagnostic criteria of the 4th national academic conference on cerebrovascular diseases [6] and were confirmed by the head CT or MRI. Exclusion criteria: experimental group excluded (1) Cerebral infarction and asymptomatic cerebral infarction were caused by infection. (2) Hemorrhage from subarachnoid hemorrhage and vascular malformation. The control group excluded: (1) Cardiovascular and cerebrovascular diseases, liver and kidney dysfunction, diabetes, hypertension, hyperlipidemia, vascular dementia and Alzheimer's disease; (2) Breastfeeding and pregnant women; (3) alcoholism and substance abused.

All included cases were collected general information, past history, clinical signs, electrocardiogram, laboratory routine, biochemical tests. Patient group: 94 patients with cerebrovascular disease in Qinghai, including 48 patients (24 males and 24 females) with a mean age of 61.39 ± 10.48 years and 46 patients with cerebral hemorrhage (20 males, 26 females), aged 63.17 ± 10.92 years. (2) Control group: 96 healthy subjects (52 males and 44 females) of Qinghai Tibetan, whose age, gender, and living altitude were matched with patients group, with an average age of 65.15 ± 9.61 years old, all from Qinghai Provincial People's Hospital between February to December 2016. None of the above subjects had a blood, and no intermarriage was found with a tribe within three generations.

According to the principle of randomized control, the subjects were randomly divided into ICH group, CI group and control group. ApoE genotypes were tested for each subject. 1) Subjects were collected fasting venous blood 3ml in EDTA anticoagulant tubes, the blood samples were stored at -80℃, after the collection of samples which were used to gene sequencing. 2) DNA was extracted by using Qiagen blood DNA kit (Wuhan China), and Quantitative Real-time PCR was carried by ABI Vii A7 Dx using Youzhiyou kit (Wuhan China).

The statistical analysis was by using SPSS 17.0 software. The classification data was expressed in terms of frequency. The frequency of the alleles was calculated as follows: ε2 = E2/2 + 1/2 (E3/2 + E4/2); ε3 = E3/3 + 1/2 (E3/2 + E3/4); ε4 = E4/4 + 1/2 (E3/4 + E4/2) [7]. Counting data was used by chi-square test, and measurement data was used by t test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of Age Distribution

In the experimental group: including 48 cases of Tibetan CI group, age was 61.39 ± 10.48 years, and 46 cases of Tibetan ICH group, age was 63.17 ± 10.92. In control group, Tibetan healthy control group has 96 patients, the aged was 65.15 ± 9.61 years. The data shown in Table 1, there were no significant difference of the age among CI group, ICH group and healthy control group (P = 0.668).

Table 1. *The age comparison of Tibetan groups.*

Group	Case	Age	χ^2	P
CI	48	61.39 ± 10.48	1.12	0.668
ICH	46	63.17 ± 10.92		
Control	96	65.15 ± 9.61		

CI group: cerebral infarction group; ICH group: cerebral hemorrhage group

3.2. Comparison of Gender Distribution

Gender distribution in experimental group: There were 24 males and 24 females in the group of Tibetan CI group, 20 males and 26 females in the ICH group. While Tibetan healthy control group included 52 males and 44 females. There was no significant difference between groups (P = 0.490). The results was shown in Table 2.

Table 2. *The gender comparison of Tibetan groups.*

Group	Male	Female	Case	χ^2	P
CI	24	24	48	1.428	0.490
ICH	20	26	46		
Control	52	44	96		

3.3. Hardy-Weinberg Balance Test

According to the Hardy-Weinberg equilibrium test, the theoretical frequency of ApoE genotypes was calculated by $(p + q + r)^2 = p^2 + q^2 + r^2 + 2pq + 2pr + 2qr$, and E2/2 = p^2, E3/3 = q^2, E4/4 = r^2, E2/3 = 2pq, E2/4 = 2pr, E3/4 = 2qr, as well as p is a frequency of ε2, q is a frequency of ε3, and r is a frequency of ε4 [8]. The Chi-square test showed that there was no significant difference between the theoretical frequency and the actual frequency in Table 3 (P = 0.54), which indicated that all of the above subjects fit the Hardy-Weinberg equilibrium and the samples included in this study were group representative.

Table 3. *Hardy-Weinberg test of ApoE gene polymorphisms.*

Genotype	Actual frequency	Theoretical frequency	χ^2	P
E2/2	0	0	6	0.54
E3/3	16	16.2		
E4/4	0	0		
E2/3	86	82.6		
E2/4	84	84		
E3/4	4	7.2		

3.4. ApoE Genotype Distribution

The ApoE genotype had been tested by using qPCR to detect the polymorphisms of the two SNPs at positions 526 and 388. When the single nucleotide at position 388 is T and position 526 is C, the genotype can be determined as E3; position 388 is T and position 526 is T, the genotype can be determined as E2; while position 388 is C and position 526 is C, the genotype can be determined as E4. After PCR amplification, the genotype was determined mainly by observing the Ct values of FAM and VIC channels as Table 4 and Table 5 shown.

Table 4. *Single Nucleotide Results Determination Table.*

	Genotype	FAM	VIC
ApoE2	ApoE 526C/C	Ct < 38	Ct ≥ 38 or No Ct
	ApoE 526C/T	Ct < 38	Ct < 38
R	ApoE 526T/T	Ct ≥ 38 or No Ct	Ct < 38
	ApoE388T/T	Ct < 38	Ct ≥ 38 or No Ct
ApoE4	ApoE 388C/T	Ct < 38	Ct < 38
	ApoE 388C/C	Ct ≥ 38 or No Ct	Ct < 38

Table 5. *Comparison of genotype results.*

	Genotype
526TT 388TT	E2/2
526CT 338TT	E2/3
526CC 388TT	E3/3
526CT 388CT	E2/4
526CC 388CT	E2/4
526CC 388CC	E4/4

3.5. The Genotype Distribution of CI, ICH and Control Group in Tibetan Population

As see in the Table 6, the result shows that E2/3 genotype was the most frequent and most common among Tibetan CI and ICH groups, followed by E2/4, E3/3, the E3/4 was most rare, the homozygous genotype of E2/2 and E4/4 were not found in our study. While in Tibetan healthy control group, E2/4 genotype was the most common, followed by E2/3, E3/4 was the least, and no E2/2, E3/3, E4/4 homozygous genotype were found. It may also be related to the small sample size may lead to the limitations of the result, or long-term hypoxia induced genotype changes.

Table 6. *Genotype distribution in CI, ICH and control group.*

	Cases	E2/2	E3/3	E4/4	E2/3	E2/4	E3/4
CI	48	0	10 (20.8%)	0	24 (50%)	12 (25%)	2 (4%)
ICH	46	0	6 (13.0%)	0	30 (65.2%)	10 (21.7%)	0
Control	96	0	0	0	32 (33.3%)	62 (64.6%)	2 (2.1%)

3.6. ApoE Allele Frequency Distribution in CI, ICH and Control

In the CI group, the ε3 allele (48.0%) was the most common, followed by ε2 (37.5%) allele, the least common was ε4 (14.6%); while in ICH group, the most common allele were ε2 (43.5%) and ε3 (45.7%), the least common was ε4 (10.9%). Among Tibetan patients with cerebrovascular disease ε3 allele was the most common. In the normal control group, ε2 (49.0%) was the most common allele, followed by ε4 (33.3%), and ε3 (17.7%) was the least common. As shown in Table 7 and Figure 1, the gene frequency distribution difference in three groups were statistically significant ($\chi^2 = 24$, $P = 0.02$).

Table 7. Frequency distribution of ApoE in CI, ICH and control group.

	ε2	ε3	ε4	χ^2	P
CI	18	23	7	15.6	< 0.01
ICH	20	21	5	15.3	< 0.01
Control	47	17	32	0.49	> 0.01

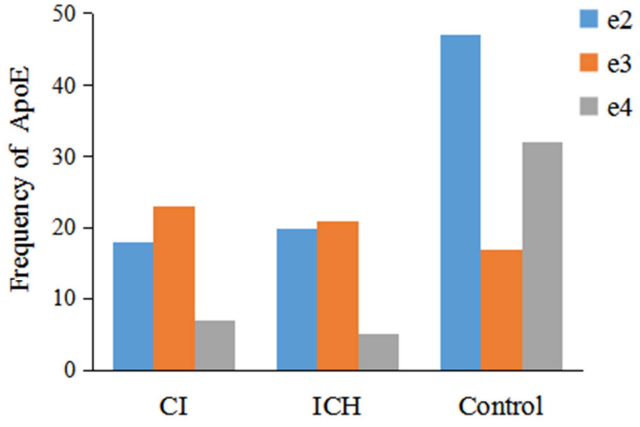

Figure 1. *Frequency distribution of ApoE alleles.*

3.7. Correlation Between Genotypes and Lipids in CI, ICH and Control Group

The serum lipoprotein research results show that in addition to TC ($P > 0.05$), the remaining three kinds of serum lipoprotein profiles were significantly different between groups, indicating a statistically significant ($P < 0.05$), shown in Table 8.

Table 8. *Comparison of serum lipoprotein profiles in groups.*

Blood lipid indicators	CI	ICH	Control	P
TG (mmol/L)	1.29±0.46	1.14±0.34	0.96±0.34	< 0.001
TC (mmol/L)	4.18±0.74	3.99±0.97	3.89±0.63	> 0.001
HDL-C (mmol/L)	0.96±0.18	0.96±0.29	1.38±0.23	< 0.001
LDL-C (mmol/L)	2.79±0.67	2.82±0.76	2.26±0.80	< 0.001

3.8. Correlation between ApoE Allele and Lipids

Comparison of blood lipids among carriers of the three alleles showed that there was a significant difference in TG ($P < 0.05$), while TC, HDL-C and LDL-C were no significant differences, shown in Table 9.

Table 9. *Comparison of blood lipids among carriers of the three alleles.*

Blood lipid indicators	ε2	ε3	ε4
TG (mmol/L)	1.18±0.38	1.01±0.40	0.93±0.28
TC (mmol/L)	3.96±0.77	3.97±0.77	4.15±0.21
HDL-C (mmol/L)	1.15±0.36	1.19±0.27	1.13±0.22
LDL-C (mmol/L)	2.30±0.90	2.64±0.81	2.54±0.50

4. Discussion

This study mainly focuses on the relationship between Tibetan cerebrovascular disease and apolipoprotein E gene polymorphism. Blood vessel related diseases have become the second most threatening human disease when the people

living in sea level, with high protein, high lipid diet. Among these cerebrovascular diseases, 59.8% are ischemic stroke and 39.3% are hemorrhagic stroke. The causes of cerebrovascular disease are varied. Hypertension, hyperlipidemia, diabetes, smoking and alcohol abuse are the indirectly risk of cerebrovascular disease. The dyslipidemia has become a hot spot of cerebrovascular disease in recent years. The study found that not only these well-known factors lead to cerebrovascular disease, but also the differences in age, gender, race, nationality was also related to this disease [7]. It has been reported that there are some differences between the ethnic groups in Yunnan ethnic minorities such as the Wa, Naxi, Bai, Yi and Xinjiang Uygur, as well as in countries of Greece, South African and Japan [8]. Qinghai is located in the northwestern of China and has lived for generations Han, Tibetan, Hui, Salar and other ethnic minorities. After the implementation of western development policy, Qinghai has achieved further improvement in both living and medical standards. In this study, we mainly discuss the relationship between cerebrovascular disease and ApoE gene polymorphism in Tibetan population and provide some evidences for studying the etiology and prevention of cerebrovascular disease in Tibetan population.

ApoE is a major apolipoprotein in the blood and locates on the chromosome 19 at region 13, band 2, about 3.7kb, including 4 exons, 3 introns. Human is mainly composed of three isomers E2, E3 and E4, which are encoded by alleles ε2, ε3 and ε4, which make up six genotypes, including E2/2, E3/3, E4/4, E2/3, E2/4, E3/4 [9]. Different amino acid sequences cause polymorphism of the gene, for example, when the 112th and 158th positions are all cysteines, the allele is E2; when both loci are arginine, the allele is E4; when 112th is arginine and 158th is cysteine, the allele is E3. It is precisely because these isomers constitute a genetic polymorphism, resulting in differences in ApoE frequency of different populations[10-11]. The physiological functions of ApoE [12-13] as follows (1) constituting lipoproteins, which are structural proteins of CM, VLDL, IDL and HDL; (2) binding to LDL receptors and apoE receptors as ligands; (3) Immune regulation, lymphocyte surface has ApoE immunomodulator receptor; (4) involved in the repair of nerve cells. Studies have shown ApoE gene polymorphism is the cause of lipid metabolism [14]. The reason is that structural changes contributed to the difference of function. ApoE polymorphism can affect plasma lipoprotein concentrations, because it can significantly upregulate receptor affinity. Receptor affinity affects the binding and uptake of CM and the decomposition process of HDL. Therefore, ApoE gene polymorphisms eventually lead to changes in circulating cholesterol levels [15-16]. Ma [17] found that compared with the patients with ϵ3 gene, the level of LDL-C was significantly increased in those who carrying ϵ4 allele, as well as LDL-C was also significantly increased in patients with E3/4 than control, which indicated that ϵ4 allele maybe affect LDL-C levels. Fu [18] found that the TC level of ApoE2 phenotype carriers in Chongqing male population is lower than other phenotypic carriers, and TC is highest in carriers of ApoE4 phenotype. Sun [19] studied the apolipoprotein gene and blood lipids in 50 patients with coronary heart disease and 156 healthy controls, and found that in the control group, the TG and LDL levels of E3 and E4 carriers were significantly higher than those in E2 carriers. While the levels of TC and LDL-C in CHD patients with E2 and E3 alleles were significantly lower than those with E4. Those studies confirmed that most of the type III hyperlipidemia patients were homozygous for ApoE2/2, and ApoE4 have normal binding activity to the receptor. The researchers also found that the plasma levels of TC and LDL-C in normal human subjects carrying ApoE2/2 or APOE3/2 genes were significantly lower than those with ApoE3/3, while which was higher in carriers of ApoE4/4 or ApoE4/3 genotypes. In conclusion, there is an opposite effect between ApoE2 and ApoE4 in affecting blood lipids, and we speculate that the E2/4 allele may be a protective factor for cerebrovascular disease.

Through this study, we found that the frequency of ε3 allele in Tibetan cerebrovascular disease is higher, the ε4 allele is less, while genotypes E2/3 is the most common, which is different from previous studies, for example, studies [20] have shown that E3/3 genotypes is the most common in ethnic minority Naxi patients with cerebrovascular disease in Yunnan, and ε3 is a protective factor for cerebrovascular disease. The study also found that ε3 was the most common in the Han population (83%-85%), followed by ε4 (5.7% -12.9%), ε2 (4%-10%), which was consistent with our findings. This may be due to the small number of cases included and the unrepresentativeness of the study. It also may be that the hypoxia and hypoxia environment is a physical stimulus to the gene mutation in Tibetan people, resulting in the majority of genes being heterozygote E2/3.

The study also found that there is a correlation between TG and ε2 alleles, suggesting that ε2 may be a susceptibility factor to promote the elevation of TG, and which provided the conditions for the occurrence of cerebrovascular diseases. This is due to the relatively poor binding capacity of ε2 to the receptor, resulting in the slow metabolism and degradation of CM and VLDL, which reduced the normal conversion of IDL to LDL, increased the concentration of HDL and decreased the concentration of LDL, eventually raised the concentration of VLDL and TG, which is in line with previous scholar's findings.

Clinical classification of ischemic stroke includes: 1, transient ischemic attack; 2, reversible neurological deficit; 3, progressive stroke; 4, complete stroke; 5, marginal infarction; 6, lacunar Cerebral infarction. The main areas covered include the internal carotid artery system and the vertebrobasilar system.

In the brain, ApoE is synthesized by astrocytes, oligodendrocytes and activated microglial cells and can participate in the uptake of lipid complexes through specialized receptors and its main function is to adjust the nerve repair, shaping and protection. ApoE can also directly participate in the redistribution of the nervous system lipid and cholesterol metabolism. In conclusion, the genetic polymorphism of ApoE causes the development of ischemic cerebrovascular diseases through the regulation of blood

lipids. Therefore, in recent years, the physiological mechanism of ApoE in the brain has become a hot spot for scholars at home and abroad. Studies have shown that ApoE4 allele plays a key role in the development of ischemic cerebrovascular disease [21]. Liu Hongjuan et al [22] found that among Han nationalities, the frequencies of ε2 and ε4 in patients with cerebral infarction were 6.9% and 9.7%, while those in the control group were 19.1% and 3.9% respectively. In ICH group, the frequencies of alleles ε2 and ε4 were 9.6% and 14.8%, respectively, which were significantly different from those in control group. Therefore, it is speculated that ApoE4 is a predisposing factor for stroke, and ApoE2 may be a protective factor of cerebral infarction. Lu Hongyan [23] who studied 50 patients with cerebral hemorrhage and 120 healthy subjects, did not find ApoE4 allele and cerebral hemorrhage had a greater correlation. Lin et al [24, 25] found that the distribution of ApoE genotypes varied among different infarct types. His research found that ApoE gene polymorphisms associated with thrombosis caused by atherosclerosis and cardiogenic cerebral embolism, had nothing to do with the lacunar infarction. Through Meta-analysis, Xie et al [26] found that the risk of atherothrombotic infarction was significantly increased in individuals with ε4 genotype, but which don't decrease in carriers with genotype ε2. A prospective study by Liu et al [27] found that population attributable risk of ApoE in cerebrovascular disease was greater than that in coronary heart disease. Foreign scholars have done related research on the occurrence of ApoE gene polymorphism and cerebrovascular disease. For example, a meta-analysis by Enzy et al [28] suggested that the ApoE4 allele is a risk factor for CI and the ApoE3 allele is protective for CI.

This study found E2/3 genotype in CI and ICH group was 50% and 65.2%, respectively. While the E2/4 genotype was 64.6% in healthy control group. Which suggested that E2/3 may be a predisposing factor for cerebrovascular disease, while the E2/4 may be a protective factor. In the Tibetan population, E3/3 was very rare, but E2/4 also has a protective effect on cerebrovascular disease, thereby reducing the incidence of cerebrovascular disease in Tibetans. Chowdhury et al [29] also showed that there is a correlation between ApoE gene polymorphism and cerebral infarction in different ethnic groups. This was quite different from the findings of Chatzistefanidis et al [30], whose study found that the E3/3 genotype was the most common in the Greek population (70%), ε3 was a common protective gene for cerebrovascular disease (83% in patients and 88% in control), followed by the ε4 alleles (11% and 6.8%, respectively) and allele ε2 (6% and 5.2%, respectively), which suggested that the ApoE2 allele may increase the risk of cerebral infarction in Greek. Misra et al [31] found that compared with the ApoE3, ApoE2 and ApoE4 alleles increased the recurrent risk of brain hemorrhage caused by hypertensive 4.3 and 11.3 times, respectively and found ApoE4 genotype was most common in patients with recurrent hypertensive intracerebral hemorrhage. We hypothesized that this may be due to the hypoxic environment in the plateau providing a favorable factor for the ApoE genotype mutation. However, the specific reasons are not yet clear and need further study.

5. Conclusion

In this study we found that the majority alleles of ApoE in Tibetan population were heterozygous E2/E3 and E2/E4, suggesting that hypoxia environment may be beneficial. The TG values in Tibetan populations varied among different alleles, suggesting that different alleles may influence lipid metabolism.

Conflicts of Interest

The authors declare that they have no competing interests. Written informed consent was obtained from each patient prior to enrolment, and the present study was approved by the ethics committee of Qinghai Province People's Hospital.

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